REMARKS

This response addresses the issues raised by the Examiner in the Office Action mailed July 26, 2004. Initially, Applicants would like to thank the Examiner for the careful consideration given this case. Claims 14, 19-21 and 24-26 have been currently amended. Thus, Claims 13-26 is presently pending. In view of the above amendments and the following remarks, Applicants respectfully submit that the presently pending claim is in form for allowance and notification of such is respectfully requested.

Abstract

The Examiner requests a corrected abstract to correct errors. To expedite prosecution of this application, Applicants have provided a corrected abstract as requested by the Examiner.

Specification

The Examiner objects to the specification under 35 U.S.C. § 132 because it introduces new matter into the disclosure.

To expedite prosecution of this application, Applicants have amended the specification as Examiner suggests with regards to the seventh paragraph regarding the phrase "after at least one separation step". However, Applicants respectfully disagree with the Examiner regarding the twelfth paragraph because the phrase "said analysis data being associated with said M liquid fraction" is supported by Claim 1. In Claim 1, the method for the multidimensional analysis of a proteome includes the step of identifying the M liquid fractions and combining the analysis data which shows that the analysis data is associated with the M liquid fractions. Thus, Applicants respectfully requests reconsideration and withdrawal of this objection.

Rejection Based Under 35 U.S.C. §112, First Paragraph

The Examiner rejects Claims 19 and 24 under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement. The Examiner argues that the claims contain subject matter not described in the specification. In particular, the Examiner has pointed out that the newly added limitation "after at least one separation step" in Claim 19 and "said analysis data being associated with said M liquid fractions" in Claim 24 do not appear in the original claims.

Applicants have amended Claim 19 to address the concerns of the Examiner. However, Applicants respectfully disagree with the Examiner regarding Claim 24 because the phrase "said analysis data being associated with said M liquid fraction" is supported by Claim 1. In Claim 1, the method for the multidimensional analysis of a proteome includes the step of identifying the M liquid fractions and combining the analysis data which shows that the analysis data is associated with the M liquid fractions. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. §112, first paragraph be reconsidered and withdrawn.

Rejection Based Under 35 U.S.C. §112, First Paragraph

The Examiner rejects Claims 13-26 under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for a compilation of data for M liquid fraction does not reasonably provide enablement for combining analysis data in any other ways. The Examiner also argues that the specification does not enable any person skilled in the art to make and use of invention. Applicants respectfully disagree.

The compilation of data obtained by three separation steps was shown as an example in order to characterize one main point of the invention. The invention is a method to lower the complexity of complex mixtures of proteins which exhibit a broad variety of combination of characteristics by separating the proteins using general protein characteristics such as shown in Claims 14 and 23 and the last paragraph on page 9 of the specification. Each final fraction obtained is uniquely characterized by fraction numbers of the separation processes that lead to the final fraction. Thus, the position of the final fractions where the protein is found contains information about their characteristics. The whole set of the final fractions give a multidimensional image of a proteome under study which may be compared to another proteome. Methods suitable for analysis of proteins within liquid fractions can be found in Claims 16-18 and under the "Identification of proteins" heading on page 6 of the specification.

Data analysis known in the art can be found in the second paragraph on page 8 of the specification. The combination of analysis data of separated proteins of a proteome with their position number in a two-dimensional gel separation and in a two-dimensional chromatography separation are also mentioned by Shevchenko et al. 1996 in the "Background of the invention" section at point 3 labeled "identification of proteins" and Opiteck et al., Analytical Biochemistry, 1998, vol. 258 in the "Background of the invention"

section at point 2 labeled "separation and detection", respectively. Thus, persons skilled in the art e.g. biochemists working in the field of proteomics, should be able to combine analysis data with positions within previous separation steps as exemplarily arranged in Table 1.

The number of liquid fractions required or desirable for each step is dependent upon the sample used with respect to the number and characteristics of the proteins and the character and quality of separation. All proteins have characteristics such as size, charge, hydrophobicity, and the sequence and structure dependent affinity to other molecules. These common characteristics are instruments suitable for separation. See Claims 14 and 23 and paragraph 5 on page 9 of the specification. The number of liquid fraction depends on the sample and separation technique. There are a multiplicity of different proteomes e.g. plasma proteome (Anderson NL, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects, Mol Cell Proteomics; 2002 Nov.; 1(11):845-67; Review Erratum in: Mol Cell Proteomics; 2003 Jan.; 2(1):50.) with completely solved proteins, cell extracts with mostly insoluble membrane proteins or urinary proteomes with huge amounts of non-protein substances. The number of proteins within a proteome can only be estimated. Therefore, necessary conditions for separation are very different and it is not possible to give a general instruction sheet.

Persons skilled in the art e.g. biochemists or biotechnologists should be able to test separation conditions for every proteome. Multiple chromatography techniques for protein separation conditions are integral parts of the course of studies for students in biosciences. For example, Applicants have conducted a Goggle search with search terms "lecture chromatography protein" yielded 17,100 hits on October 4, 2002. The following are only three of the 17,100 hits of the Google search:

1) http://www.bio.cmu.edu/courses/03231/LecF04/Lec16/lec16.pdf (Mellon College of Science)

2) http://www.sci.sdsu.edu/TFrey/Chem365/Proteins/ProteinsChem365.html (San Diego State University)

3) http://ntri.tamuk.edu/hplc/hplc.html#Introduction (Natural Toxins Research Center at Texas A&M University – Kingsville)

In addition, one often used textbook for students containing a chapter on chromatography of proteins is Fundamental of Biochemistry by D. Voet, J.G. Voet and C.W. Pratt Wiley & Sons, Inc. 1998.

Also, Applicants have conducted a Goggle search with search terms "laboratory chromatography protein student" yielded 39,100 hits on October 4, 2002. The following are only three of the 39,100 hits of the Google search:

1) http://www.ncsu.edu/biotechnology/courses/bit464_564.php (NC State University, Biotechnology)

2) http://www.kckcc.edu/syllabi/chem0251o.psp (Kansas City Kansas Community College)

3) http://www.okcc.edu/biotech/document/btlab2.html (OKC Community College)

Furthermore, there are laboratory notebooks for persons skilled in the art giving advice for protein separation, e.g. Current Protocols in Protein Science by John E. Coligan et al., editorial board, John Wiley & Sons, Inc. 1995-2002, chapter 8. Thus, the present invention does enable one skilled in the art to make or use the invention. Accordingly, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

Rejection Based Under 35 U.S.C. §112, Second Paragraph

The Examiner rejects Claims 13-24 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The Examiner also argues that the specification does not enable any person skilled in the art to make and use of invention. Applicants respectfully disagree.

The present invention discloses a method for the multidimensional analysis of a proteome. The main points of the invention are first to lower the complexity of a proteome where the proteome is subjected to n different separation processes, n>2 in a way that each of the liquid fractions of the previous separation is subjected to subsequent separating steps. See Claim 13. The protein characteristic that are used for this purpose are disclosed in Claim 14 and paragraph 5 on page 9 of the specification. An example of the three separation processes is shown in the "Description of the Preferred Embodiments" section on page 9 of the specification.

Second, various methods in the art for protein determination and identification within the resulting liquid fractions are shown in Claims 15-18 and under "Identification of the proteins" section of the specification on page 6. The result consists of identifiers and

quantifiers for the protein present in each fraction. Liquid fractions allow a simpler analysis of protein than compared with 2-dimensional electrophoresis. See "Separation and detection" section of the specification on page 4. Moreover, utilization of characteristics of native proteins as enzyme activity after separation is possible in liquid fractions but excluded for 2-dimensional electrophoresis.

Third, as stated above, every resulting fraction with its protein content has a unique set of fraction numbers consisting of fraction numbers corresponding to the sequence of separation processes. Thus the position of a protein contains information about its characteristic and all these information provide a multidimensional image of the whole proteome which can be compared with another proteome. Methods suitable for analysis of proteins within liquid fractions can be found in Claims 16-18 and under the "Identification of proteins" heading on page 6 of the specification. Data analysis known in the art can be found in the second paragraph on page 8. The combination of analysis data of separated proteins of a proteome with their position number in a two-dimensional gel separation and in a two dimensional chromatography separation are also mentioned by Shevchenko et al. 1996 in the "Background of the invention" section at point 3 labeled "identification of proteins" and Opiteck et al., Analytical Biochemistry, 1998, vol. 258 in the "Background of the invention" section at point 2 labeled "separation and detection", respectively. Thus, persons skilled in the art e.g. biochemists working in the field of proteomics, should be able to combine analysis data with positions within previous separation steps as exemplarily arranged in Table 1. Further, Claim 24 states that analysis data are assembled in a database where data analysis data is associated with the M liquid fractions.

In one embodiment of the invention, the results of the three separation processes are 10,000 fractions with their respective position. See Description of the Preferred Embodiments on page 9 of the specification. This requires the examination of at least 10,000 samples with methods discloses in Claim 15-18 and under "Identification of the proteins" section of the specification on page 6. Each further separation step amplifies the number of resulting fractions exponentially. Therefore, a way of permitting high throughput of sample is required. Claims 19-20, and 25-26 give the preferred collection and storage mode within microplate format. The use of such a format is a pre-requisite for application of high throughput liquid handling system and automation of liquid sample handling. Claim 23 refers to parallel chromatography for the second and all following separation steps in order to avoid a time consuming separation sequence and to avoid changes with time within the protein samples during separation.

In regards to Claim 13, Claim 13 discloses τ different analysis processes to be applied to the resulting M fraction. This analysis process yields qualitative and quantitative analysis data where the qualitative analysis data are used as identifiers for characterizing the proteome according to the state of the art in Claims 13 and 24 and under "Data Analysis" section on page 8 of the specification. Identification and quantification can be done in one process e.g. in an immunological process or determination of catalytic activity which gives identity and amount of protein. See Claims 15 and 16. Claim 13 refers to an unspecified protein determination processes. See Claim 16. Therefore, there are two types of quantitation of the liquid fractions.

In regards to Claim 14, Applicants have amended Claim 14 to list the processes in the alternative and clarify that methods which separate according to the affinity of the protein with respect to specific ligands are separate from methods which separate according to the affinity of the protein with respect to specific antibodies. The affinity of other molecules to a protein could be based on binding sites for specific ligands e.g. cofactors as nucleotides, substrate binding sites, specific inhibitors bind at substrate or at regulatory binding site, and lectins for glycosylation sites. The specificity is often grouped specific and therefore suitable to separate different proteins with one ligand for conserved binding site. Antibodies are immunglobins developed against at least one recognition site of the respective protein. If they do not show cross reactivity, they will be specific to one protein. Thus, Claim 14 differentiates between ligands and antibodies.

In regards to Claim 16, the first part of Claim 16 states "wherein methods for nonspecific determination of protein concentration with different sensitivities..."refers to Claim 13, line 11 where nonspecific determination can be applied to all proteins within fractions. The second part of Claim 16 states "...and/or quantitative determination methods for determining specific catalytic activities and/or quantitative immunological methods and/or quantitative binding assays..." refers to Claim 13, line 10 where specific determination as e.g. catalytic activity can be applied to at least one protein as well as for proteins which are recognized in a group specific manner. The method specific for a unique protein is also equivalent to an identifier if it detects this protein.

Claims 19, 20, 21, 25 and 26 have been amended as Examiner suggests. The phrases "two dimensional multiple vessel system with the layout of microtitration plates" and "in a defined grid of microtitration technology" in Claim 19, 20 and 21, respectively, have been replaced with the phrase "d x 96 well microtiter plates, where d is an integer>0." Also, in

Claim 25 and 26, the phrase "n*96 grid of microtitration technology" is replaced with the phrase "d x 96 well microtiter plates, where d is an integer>0."

Claim 24 relates to Claim 13 that states that after gathering the analysis data of M fractions, the information content of the n-dimensional image of the proteome is increased by combination of identifiers and quantifiers with the positions of the fraction according to the separation sequence. See "Data analysis" section of the specification on page 8. Claim 24 states "wherein the analysis data for n-dimensional image of protein are assembled in a database, said analysis data being associated with said M fractions." Therefore, Claim 24 has been amended to clarify that the analysis data consists of quantifiers and identifiers. This is combined with the position of the respective fraction within the separation. See Table on page 10 of the specification. Accordingly, Applicants respectfully request withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

Rejection Based On Opiteck Under 35 U.S.C. § 102 (b)

The Examiner rejects Claims 13-14, 16-19, 21 and 23-24 under 35 U.S.C. § 102 (b) as being anticipated by Opiteck et al. (Analytical Biochemistry, May 1998). Applicants respectfully traverse this rejection.

To establish obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art. MPEP § 2143.03. Opiteck describes the operation of a cell lysate of E. coli with over expressed recombinant protein by an online combination of size exclusion chromatography and reverse phase columns (see below Figure 1). Opiteck combines identification data with their position at their respective separation times. Separation by RP columns is performed pseudo parallel, because at every time only one column is used for separation while the other column is trapping eluate (see below figure 2).

In contrast, the present invention discloses a method for the multidimensional analysis of a proteome comprising the steps of subjecting the proteome to a number n of different separating processes for n>2 under standardized conditions in such a way that each of the liquid fractions m_1 obtained in a separating step supplies m_2 liquid fractions in a subsequent separating steps step, wherein, after n separating steps, there are $m_1 * m_2 * \ldots m_n = M$ liquid fractions; identifying said $m_1 * m_2 * \ldots m_n = M$ liquid fractions by τ different analysis processes qualitatively and/or quantitatively by identification processes, and determining said liquid ratio fractions quantitatively by known quantification processes; and after combining

the analysis data, obtaining an n-dimensional image of the proteome which is characterized by identifiers and quantifiers and by the position in the n-dimensional data space. The present invention discloses an offline procedure, which produces necessarily fractions during each separation step and these fractions are subjected to the separation described above. See Claim 13 and the "Description of the Preferred Embodiments" section on page 9 of the specification. An online separation procedure generally constrains the choice of separation methods, separation time, and sample amount. The separation time decreases exponentially from separation 1 (340 min.) to separation 2 (3.4 min).

Although Opiteck discusses possible improvement for handling a proteome with online coupled columns, Opiteck proposes larger columns and other separation phases for further improvement of resolution but not additional separation dimension for the protein which is an obvious solution for the resolution problem. See page 359. It seems very unlikely, that the very short separation times of their second dimension will be compatible with a third online separation dimension because of the above mentioned exponential decrease of separation time. Furthermore, Opiteck concludes on the basis of their results that their two-dimensional separation could not achieve the resolution according to the state of the art of the two-dimensional electrophoresis. See page 359. A further disadvantage of Opiteck is the sensitivity to failure of a single component within the series of separation devices because one failure can lead to the loss of the whole experiment.

In contrast, the procedure described by the present invention enables control and adjustment after every separation step. The present invention solves the indicated problems by fractionation after each separation step n>2 and by the use of parallel separation steps to obtain acceptable separation times. See Claim 13, 23 and "Description of the Preferred Embodiments" section on page 9 of the specification. In the present invention, the management of huge amounts of samples by established liquid handling methods and use of formats, such as microplates are well suitable to automation and ensure an acceptable effort for proteome sample separation and for compatibility with generally used analysis techniques. See Claims 19-21 and 25-26. Combination of analysis data with their respective positions in our separation sequence and fractions allow the comparison of proteomes with each other. Thus, the present invention combines more than two separation dimensions for high resolution consequently in liquid phase with acceptable effect, comprehensive analyses and data combination in the state of art for proteins within the resulting fractions.

Opiteck does not specifically teach or disclose a method for the multidimensional analysis of a proteome where the offline procedure produces fractions during each separation step and are subjected to different separating processes. Further, Opiteck does not disclose more than two additional separation dimensions for proteins. Thus, Opiteck does not disclose, teach or suggest the method for the multidimensional analysis of a proteome of the present invention. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 102 (b) be reconsidered and withdrawn.

Rejection Based On Blackstone Under 35 U.S.C. § 102 (b)

The Examiner rejects Claims 13-18 and 23-24 under 35 U.S.C. § 102 (b) as being anticipated by Blackstone et al. (TITCH, March 1999). Applicants respectfully traverse this rejection.

Applicants assume that Examiner meant to cite Blackstock et al. instead of Blackstone et al. as the author of TIBTECH, March 1999. Applicants' literature search did not come up with any hits for Blackstone et al. Thus, Applicants will address this rejection in regards to Blackstock.

For a rejection to be sustained under 35 U.S.C. § 102 (b) each and every element of the claimed invention must be disclosed in the cited prior art. Blackstock discloses a pre-fractionation of tagged cellular proteins followed by the main separation step, i.e. the two-dimensional gel electrophoresis. However, Blackstock does not disclose a method for the multidimensional analysis of a proteome which produces liquid fraction during each separation step and the fractions are subjected to different separating processes where liquid fractions are identified by different analysis processes qualitatively and/or quantitatively by identification processes, and liquid ratio fractions are determined quantitatively by known quantification processes, and after combining the analysis data, obtaining an n-dimensional image of the proteome which is characterized by identifiers and quantifiers and by the position in the n-dimensional data space.

As stated above, in contrast, the present invention discloses a method for the multidimensional analysis of a proteome comprising the steps of subjecting the proteome to a number n of different separating processes for n>2 under standardized conditions in such a way that each of the liquid fractions m_1 obtained in a separating step supplies m_2 liquid fractions in a subsequent separating steps step, wherein, after n separating steps, there are $m_1 * m_2 * \ldots m_n = M$ liquid fractions; identifying said $m_1 * m_2 * \ldots m_n = M$ liquid fractions by τ

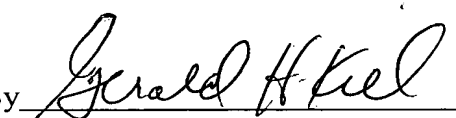
different analysis processes qualitatively and/or quantitatively by identification processes, and determining said liquid ratio fractions quantitatively by known quantification processes; and after combining the analysis data, obtaining an n-dimensional image of the proteome which is characterized by identifiers and quantifiers and by the position in the n-dimensional data space. The present invention also does not require tagged proteins. The disadvantages of the traditionally used two-dimensional gel electrophoresis e.g. restriction of sample volume used, separation is limited to two dimensions,...etc. See under "Separation and detection" heading on page 4 of the specification. In addition, the present invention describes a procedure that produces necessarily liquid fraction during each separation step and the fractions are subjected to the separation described in Claim 13 and in the "Description of the Preferred Embodiments" on page 9 of the specification where all analysis steps uses proteins within liquid fractions according to the state of the art. See Claims 15-18 and under the "Identification of proteins" heading on page 6 of the specification. Data storage with quantifiers and identifiers are related to the respective position of the separation in claim 24, which is even described in Blackstock.

Blackstock does not disclose a method for the multidimensional analysis of a proteome where the proteome is subjected to a number n of different separating processes for $n>2$ under standard conditions in such a way that each of the liquid fractions m_1 obtained in a separating step supplies m_2 liquid fractions in a subsequent separating steps step where after n separating steps, there are $m_1 * m_2 * \ldots m_n = M$ liquid fractions, then the M liquid fractions are identified by τ different analysis processes qualitatively and/or quantitatively by identification processes, and the liquid ratio fractions are quantitatively determined by known quantification processes. Since Blackstock does not disclose these features of the present invention, Blackstock does not disclose each and every claim element of the claimed invention. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 102 (b) be reconsidered and withdrawn.

The above amendments and accompanying remarks address each and every concern raised by the Examiner in the Office Action. Based on these clarifying amendments, Applicant believes that all claims of the present invention are now in condition for final allowance. As outlined above, each of these amendments is fully supported throughout the specification, and no new matter is introduced by these amendments. If the Examiner feels that any issues remain outstanding, the Examiner is encouraged to contact Applicant's attorney at the contact information below.

Respectfully submitted,

Dated: November 24, 2004

By 
Gerald H. Kiel
Reg. No. 25,116

REED SMITH LLP
599 Lexington Avenue
29th Floor
New York, NY 10022-7650
(212) 521-5400

Attorney for Applicant